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PRINCIPAL INVESTIGATOR: Keith R. Solomon

CONTRACTING ORGANIZATION: Children's Hospital Corporation, The
Boston, MA 02115-5724

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14. ABSTRACT Prostatic adenocarcinoma (PCa) is the most common form of non-cutaneous cancer and second most lethal cancer in American men but demonstrates tremendous disparity in both incidence and severity between African American men (AAM) and Caucasian men (CM). We have identified <i>prostatic intratumoral steroidogenesis</i> as a biological factor that may explain some or much of the disparity in lethal PCa rates between AAM and CM. We proposed testing this hypothesis by examining intratumoral steroidogenesis in the prostates of men following radical prostatectomy and <i>in vivo</i> model systems. In this project period we have finished our initial round of <i>in vivo</i> modeling and have demonstrated that hypercholesterolemia contributes to increased androgen levels and higher levels of nuclear localized AR as well as prostate tumor growth (as reported in last year's progress report) in our model mimicking the human patient situation in which androgen deprivation therapy (castration) is applied after tumor initiation. Blinded end point testing is ongoing and we anticipate further strengthening the statistical significance of our findings as we analyze more tumors. The final data are anticipated to reveal that, as hypothesized, hypercholesterolemia contributes to faster relapse after castration and increases intratumoral steroidogenesis.					
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Introduction:

Prostatic adenocarcinoma (PC) is the most common form of non-cutaneous cancer and second most lethal cancer in American men but demonstrates tremendous disparity in both incidence and severity between African American men (AAM) and Caucasian men (CM). AAM have an incidence rate of 231.9 PCs/100,000 men whereas CM have an incidence rate of 146.3 PCs/100,000 men, a ratio of 1.58 AAM/CM, while the mortality rate for AAM is 56.3/100,000 and for CM is 23.6/100,000 men, a ratio of 2.39 AAM/CM⁽¹⁾. These data show that AAM not only have a disproportionate incidence rate, but their mortality rate is almost 2.5X higher, suggesting that AAM are more likely than CM to have their cancer progress to advanced, fatal disease. The reasons for the high degree of disease burden in AAM are unknown, but may stem from biological, economic, psychological and sociological origins. Low socioeconomic status, absence of health insurance, poor access to health care, and lack of a regular primary care physician are substantial barriers to PC screening and early diagnosis⁽²⁾. These factors are likely responsible for some of the unequal disease burden between AAM vs. CM, but it is also apparent that there is a biological basis for the PC risk discrepancy. We have identified *prostatic intratumoral steroidogenesis* as a biological factor that explains some or much of the disparity in lethal PC rates between AAM and CM. In addition, circulating cholesterol has been identified in pre-clinical and epidemiological studies as a likely promoting factor in PC progression⁽³⁻¹⁷⁾. We hypothesize that serum cholesterol level affects tumor steroidogenesis by both serving as a rate-limiting metabolite precursor of intratumoral androgen synthesis, as well as a steroidogenic pathway agonist. We predict that we will find higher androgen levels in the tumors of men with higher levels of cholesterol. We further hypothesize that for any given serum cholesterol level, AAM will have higher tumor steroidogenesis. Using *in vivo* model systems we are testing whether therapeutically targeting cholesterol will reduce intratumoral steroidogenesis. This project specifically focuses on the effectiveness of cholesterol targeting as a means of delaying disease progression in the context of castration resistant prostate cancer, which is uniformly fatal.

Body:

Task 1. Determine whether there is a disparity in level of steroidogenic enzymes and androgens between the prostatic tumors of African American and Caucasian men (1-24 months). This task requires recruitment of patients, collecting patient serum, serology including cholesterol measures, radical prostatectomies or excision of metastatic lesions (Duke site), shipment of frozen tissue to the Solomon lab (Children's Hospital Boston, CHB, site), for analysis of androgens and steroidogenic enzymes.

To accomplish task 1 we spent 1.5 years attempting to create a human subjects protocol at Duke University Medical Center and the Durham VA and despite their uncollegial obdurateness at Duke we were successful in creating the protocol. Unfortunately Duke placed in the way new obstacles that made getting testable samples from them very difficult. Consequently, after consultation with DoD staff, we were granted approval to alter our SOW (revised SOW is indicated below) to only include only patient samples obtained through the Durham VA. We have an approved protocol from the VA (see below), the patient demographics at the VA are better for the purpose of our study than the demographics at Duke, and we will be able to obtain the same number of patient samples as we initially proposed.

Revised Statement of Work (SOW):

Task 1. Determine whether there is a disparity in level of steroidogenic enzymes and androgens between the prostatic tumors of African American and Caucasian men (1-24 months). This task requires recruitment of patients, collecting patient serum, and serology including cholesterol measures, radical prostatectomies or excision of metastatic lesions (Duke site), shipment of frozen tissue to the Solomon lab (Children's Hospital Boston, CHB, site), for analysis of androgens and steroidogenic enzymes.

- 1a. Recruitment of patients (30 African American and Caucasian men/annum), patient histories and collection of serum (Duke site) (months 12-36).
 - 1b. Removal of cancer tissue (Duke site) (months 12-36).
 - 1c. Serology (Duke site) (months 12-36).
 - 1d. Shipment of froze tissue to Children's Hospital Boston (months 12-36).
 - 1e. Analysis of androgens (CHB site) (months 12-36).
 - 1f. Analysis of steroidogenic enzymes (CHB site) (months 12-36).
 - 1g. Statistical analysis of the racial disparity in steroidogenesis and its association with cholesterol level (Duke site) (months 30-36).
 - 1h. Preparation of materials for data dissemination (months 30-36).
- Task 2. Using in vivo model systems we will determine if therapeutically targeting cholesterol alters intratumoral steroidogenesis. This task requires placing mice on specialized diets that raise or reduce circulating cholesterol levels, implanting xenograft tumors, measuring tumor growth, and determining the effect of cholesterol raising and reducing on tumor growth and intratumoral steroidogenesis (CHB site) (months 1-36):
- 2a. Determine whether castration and/or hyper- and hypocholesterolemia affects steroidogenesis in prostatic tumors of human origin (months 1-36).
 - 2ai. Place mice on standard low fat, no cholesterol diet for 2 w (280 mice) (starting in months 2-3 of project depending on availability of mice, drugs and diets). Month one is reserved for purchasing of materials.
 - 2aai. Randomize mice to 5 cholesterol raising or lowering diets for 2 w.
 - 2aiii. Castrate 1/2 the mice.
 - 2aiiii. Implant LNCaP C81 and C33 xenografts, respectively.
 - 2av. Continue mice on diets (2-3 w).
 - 2avi. Collect serum (biweekly).
 - 2avii. Measure tumors growth (2-3 w).
 - 2aviii. Analysis of steroidogenesis, proliferation, apoptosis, angiogenesis, and AR activation in harvested tumors (months 4-30).
 - 2aviii. Serology (months 3-30).
 - 2ax. Analysis of data (months 7-36).
 - 2axi. Preparation of materials for data dissemination (months 24-36).
 - 2axii Place mice on standard low fat, no cholesterol diet for 2 w (280 mice) (starting in months 11-14 depending on availability of mice, drugs and diets).
 - 2axiii. Randomize mice to 5 cholesterol raising or lowering diets + abiraterone for 2 w.
 - 2axiii. Castrate 1/2 the mice.
 - 2axiv. Implant LNCaP C81 and C33 xenografts, respectively.
 - 2axv. Continue mice on diets (2-3 w).
 - 2axvi. Collect serum (biweekly).
 - 2axvii. Measure tumor growth (2-3 w).
 - 2axviii. Analysis of steroidogenesis, proliferation, apoptosis, angiogenesis, and AR activation in harvested tumors (months 13-30).
 - 2axviii. Serology (months 12-30).
 - 2axix. Analysis of data (months 16-36).
 - 2axx. Preparation of materials for data dissemination (months 24-36).
- 2b. Determine whether cholesterol reduction slows PCa relapse after castration (months 18-36).
 - 2bi. Place mice on standard low fat, no cholesterol diet for 2 w (210 mice are required).
 - 2bii. Randomize mice to 5 cholesterol raising or lowering diets for 2 w.
 - 2biii. Implant LNCaP C33 xenografts.
 - 2biii. Continue mice on diets (7 d).
 - 2bvi. Collect serum (biweekly).

- 2bvii. Measure tumor growth (7 d).
- 2bviii. At approximately day 7 castrate 2/3 of the mice.
- 2bviii. All mice will be continued on their diet regimen (7d to 6m).
- 2bx. All of the intact and 50% of the castrated mice will be harvested on postcastration day 7, the remaining castrates will be harvested 6 m post castration (7d to 6m).
- 2bxi. Measure tumor growth (7d-6m).
- 2bxii. Remove tumors for analysis of steroidogenesis, proliferation, apoptosis, angiogenesis, and AR activation (≈months 19-26).
- 2bxiii. Serology (months 18-30).
- 2bxiii. Analysis of data (months 20-36).
- 2axv. Preparation of materials for data dissemination (months 24-36)

1.0 STUDY DESIGN

This is a prospective study with no clinical intervention. Eligible patients will include those undergoing a radical prostatectomy regardless of disease risk or men undergoing excision of tissue for CRPC progression. Accrual will occur at the Durham VA. We anticipate enrolling 120 men undergoing a radical prostatectomy and 20 men undergoing excisional biopsy for CRPC progression over 2 years. After providing written consent, a blood sample, anthropomorphic measures, and basic medical history will be obtained prior to surgery. At the time of surgery, a sample of the excised tissue (either radical prostatectomy or excisional biopsy tissue) will be frozen and sent to Dr. Keith Solomon at Boston Children's Hospital for analyses to measure tissue androgen levels and expression of steroidogenic enzymes. All tissue samples will be sent to Dr. Solomon and will be labeled only with unique subject number and date of surgery. Results from the tissue analyses will be returned to the Durham VA for statistical analyses.

2.0 ELIGIBILITY CRITERIA

2.1 Inclusion Criteria:

1. Pathologically confirmed adenocarcinoma of the prostate
2. Elected primary radical prostatectomy or undergoing excision of tissue for CRPC progression including TURP
3. Race is either African-American or Caucasian
4. Evidence of a personally signed and dated informed consent document indicating that the subject has been informed of all pertinent aspects of the study.

2.2 Exclusion Criteria for Men in the Radical Prostatectomy Cohort:

1. History of ever receiving hormone or antiandrogen therapy (e.g. finasteride, dutasteride, Avodart)
2. Prior prostate radiotherapy (external beam or brachytherapy) or cryotherapy
3. Patient did not have surgery

2.3 Exclusion Criteria for Men Undergoing Excision of Tissue for CRPC Progression:

1. Unable to provide written informed consent.
2. Patient did not undergo excision of tissue for CRPC progression including TURP.

3.0 STUDY PRECEDURES

3.1 Visit Schedule:

Table 1: Evaluation and visit schedule

Examination	Screen	Study Visit	Tissue Collection
	D-60 to -1	D-60 to -1	Day of Surgery (D0)
Consent	X	X	
Eligibility	X	X	
Medical History and Demographics		X	
PSA		X	
Anthropometric Measures ¹		X	
Testosterone		X	
Free Testosterone		X	
Lipid Panel		X	
SHBG		X	
Prostatectomy or excision of CRPC progression ²			X

1 Height, weight, and waist circumference will be measured and collected

2 With tissue procurement for molecular assessments

3.2 Screening and Study Visit:

A HIPAA waiver will allow the study coordinator to perform the initial screening for eligibility among men undergoing a radical prostatectomy or excisional biopsy for CRPC progression. The study coordinator will use the computerized medical records system (CPRS) to ensure patients meet the inclusion/exclusion criteria stated in the protocol. Upon determining patient eligibility, the study coordinator will first speak with the patient at his pre-op appointment and attempt to consent him. If the subject is interested in participating, he can either complete the study procedures that day or the study coordinator can schedule a screening visit in the near future, as long as it is within our window (Table 1). Documentation of the consent process and a copy of the signed consent will be maintained in the patient's medical record.

All study procedures are permitted within the window frame indicated in Table 1. The screening and study visit may be combined as the same visit.

The following procedures will be completed for this study:

1. Anthropometric measures: Height, weight, and waist circumference measurements and will be completed by the study coordinator.
2. Blood collection: Blood will be drawn and processed at the VA for analysis of testosterone, free testosterone, PSA, lipid panel, and SHBG.
3. Medical history and demographics: Obtain medical history and demographic information from patient and via electronic medical records. This will be recorded on a CRF and will be kept in a locked file cabinet in Dr. Freedland's research office.

4. Medical record follow-up: Outcome data (i.e. PSA recurrence, time to recurrence, additional treatment, metastatic disease, etc.) will also be collected. This involves research staff following patients through electronic medical records indefinitely.

3.3 Day of Surgery: The following will be performed on the day of surgery:

1. Either radical prostatectomy or biopsy for excision of tissue for CRPC progression, which the patient is scheduled for – this is not research.
2. Tissue collection at the time of surgery/biopsy for pathologic assessment and research assessment.

4.0 TISSUE COLLECTION

4.1 Prostate Tissue Collection: Coded, frozen prostate samples will be sent to Dr. Keith Solomon at Boston Children's Hospital. The prostate tissue sample will be obtained immediately after removal of the prostate or excisional biopsy. This will be done in conjunction with the Pathology Department at the Durham VAMC as not to interfere with appropriate pathological interpretation of the specimen for clinical purposes. This tissue will be sent in batches by an overnight carrier to Dr. Keith Solomon at Boston Children's Hospital for analysis. Samples will be stored at Dr. Freedland's laboratory at Duke in the Medical Sciences Research Building until shipped to Dr. Solomon (stored <90 days). Unused samples will be returned to the Durham VA and stored either at the VA or at Duke in Dr. Freedland's laboratory under an off-site tissue bank waiver.

4.2 Excisional Biopsy Tissue Collection: A portion of tissue biopsied from CRPC lesions will be taken and placed in a tube and snap frozen. Similar to the prostate tissue collection, we will also send this tissue via an overnight carrier to Dr. Keith Solomon at Boston Children's Hospital for analysis. All samples shipped will be tracked by their respective tracking number.

Frozen specimens will be shipped on dry ice to the following address:

Dr. Keith Solomon Children's Hospital Boston
Department of Urology
Enders 10
61 Binney st
Boston, MA 02115

5.0 TISSUE ANALYSIS

Dr. Solomon will measure the level of steroidogenic/cholesterol sensitive enzymes using qPCR and western blotting: PSA, CYP17A1, CYP11A1, STaR, HSD3B1/2, HSD17B3, AKR1C1/2/3, 5RD5A1/2, HSD17B10, CYP19A1, ABCA1, ABCG1, ABCA7, CYP27A1 CYP7B1, LDLR & SR-B1, acyl-CoA cholesterol acyl transferase (ACAT), and HMG-CoA reductase. From the same tissue samples, Dr. Solomon will use mass spectrometry (MS) to measure tumor tissue levels of androstenedione, T, DHT, DHEA, and androstenediol. Finally, he will use immunofluorescence to analyze tumors for nuclear localization of the androgen receptor (AR).

Task 2. Using *in vivo* model systems we will determine if therapeutically targeting cholesterol alters intratumoral steroidogenesis. This task requires placing mice on specialized diets that raise or reduce circulating cholesterol levels, implanting xenograft tumors, measuring tumor growth, and determining the effect of cholesterol raising and reducing on tumor growth and intratumoral steroidogenesis (CHB site) (months 1-36).

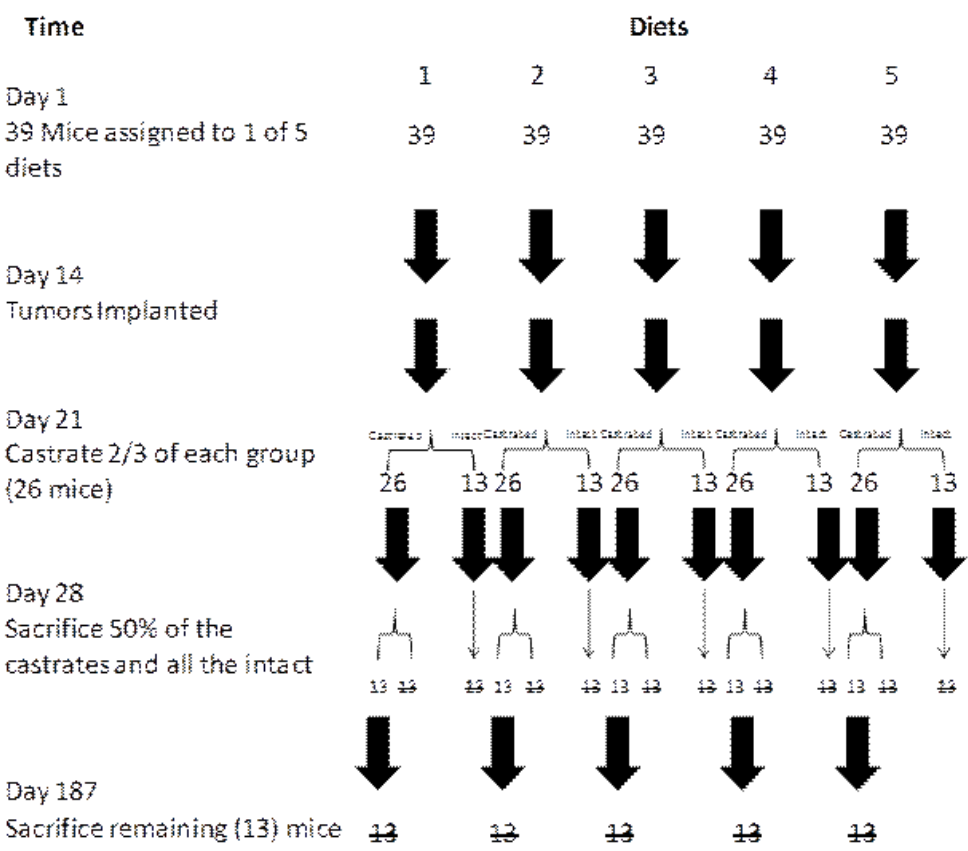


Figure 1. Diet-castration experiment scheme.

In castrated mice a high fat, high cholesterol diet increases AR activation in PCa tumors. Based on the scheme represented in Fig 1 we analyzed the effects of diet on AR activation and androgen levels in mice that were fed either our LFNC or our HFHC diet, had LNCaP PCa tumors implanted and then were subsequently castrated. Although we had initially planned to carry out the experiment for several months (see Fig 1), we halted the experiment after 6 w due to a high attrition rate. At sacrifice, tumors were removed and analyzed for steroid/androgen levels by mass spectrometry (Fig 2) and AR nuclear localization by immunohistochemistry (Fig 3). First, to ensure that we could accurately measure the very low levels of androgens found in the tumors of castrated mice we compared the levels of androgens (testosterone and DHT) in prostate tumors (LNCaP) implanted in male mice that were subsequently castrated vs. the level of androgens found in breast tumors (MDA-MB-231) implanted in female mice (Fig 2A). This analysis suggested that we could, indeed, measure the low androgen levels present in PCa tumors implanted in male mice that were subsequently castrated. Once that we had established that the we could make statistically significant measures of androgens at very low levels we analyzed the effect of diet on the intratumoral steroid/androgen levels in male mice implanted with human prostate tumors, which were fed either the LFNC or the HFHC diet, and then were castrated (Fig 2 B, C). This analysis demonstrated that the intratumoral levels of progesterone and pregnenolone in mice fed the HFHC diet were lower than that of mice fed the LFNC diet (Fig 2B). This finding is consistent with the fact that these steroids are CYP17 substrates, and are anticipated to be at lower levels in the presences of higher levels of CYP17 activity, which we hypothesize is leading to increased tumor growth in the presence of castrate levels of circulating androgens. Analysis of testosterone and DHT also showed higher intratumoral levels of these steroids trending in the mice fed the HFHC diet, although we have not yet analyzed sufficient tumors to obtain

statistical significance (further analysis of additional tumors is ongoing) (Fig 2C). Consistent with higher levels of androgens in the tumors grown in castrated mice fed the HFHC diet, analysis of AR nuclear localization strongly suggests that in the castrate environment a HFHC diet increases the levels of nuclear AR (Fig 3). In total, our new data suggest that our hypothesis has survived challenge and that a HFHC diet promotes PCa tumor growth in the castrate environment through increased androgen synthesis leading to AR translocation and activation.

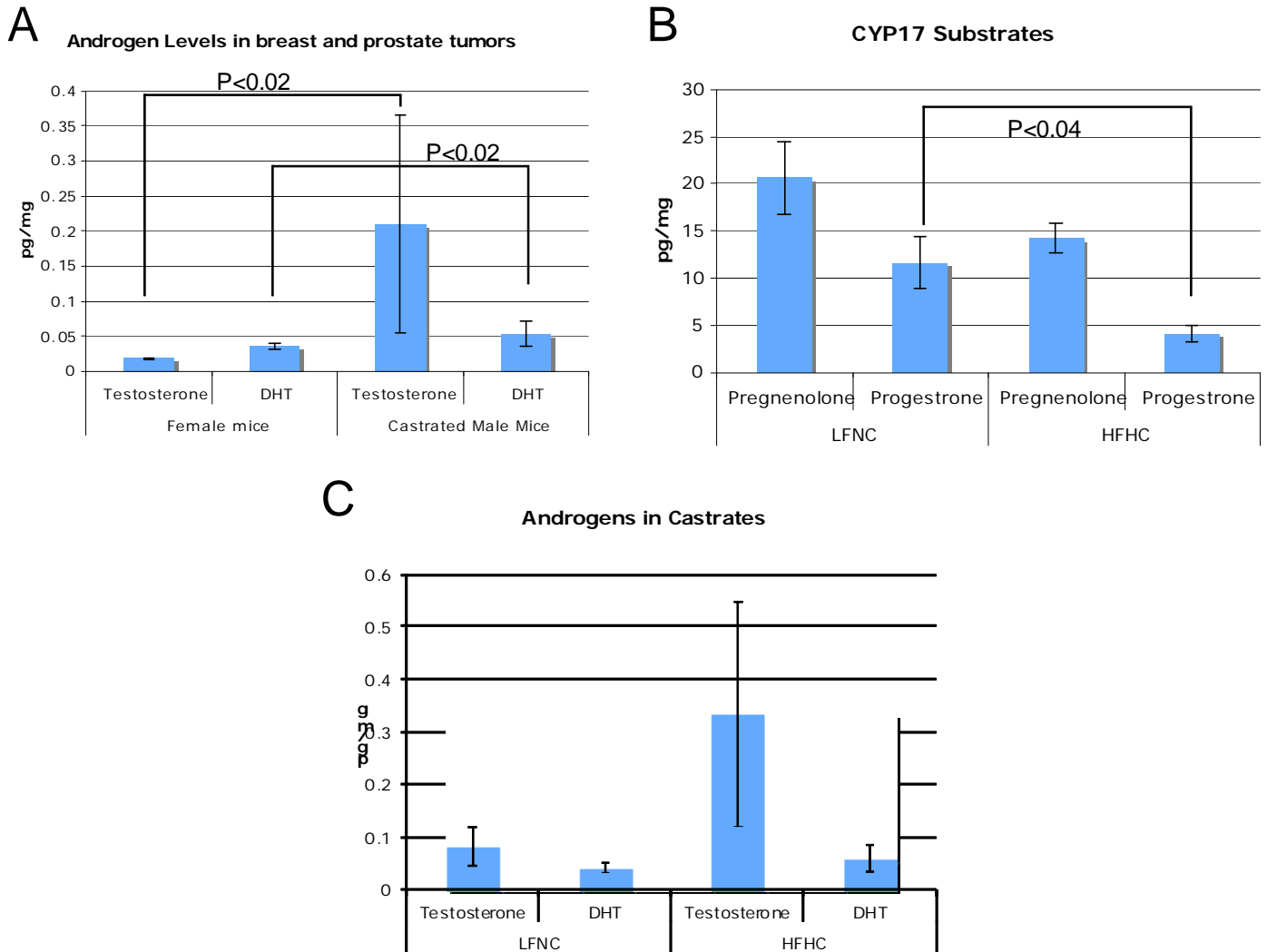


Figure 2. Steroid/androgen level analysis in xenograft tumors of human origin. A. Androgens (testosterone and DHT) were measured by mass spectrometry in MDA-MB-231 breast tumors implanted in female mice and in prostate tumors (LNCaP) implanted in male mice that were subsequently castrated. Data are plotted as pg androgens/mg tumor tissue vs. group \pm SEM (n=10-20/group). B. CYP17 substrates (pregnenolone and progesterone) were measured in the LNCaP tumors as described above. Data are plotted as pg androgens/mg tumor tissue vs. group \pm SEM (n=5/group). C. Androgen levels were measured in the LNCaP tumors as described above. Data are plotted as pg androgens/mg tumor tissue vs. group \pm SEM (n=10/group). The Student's t test was used to determine significance in all cases and $p < 0.05$ is considered significant.

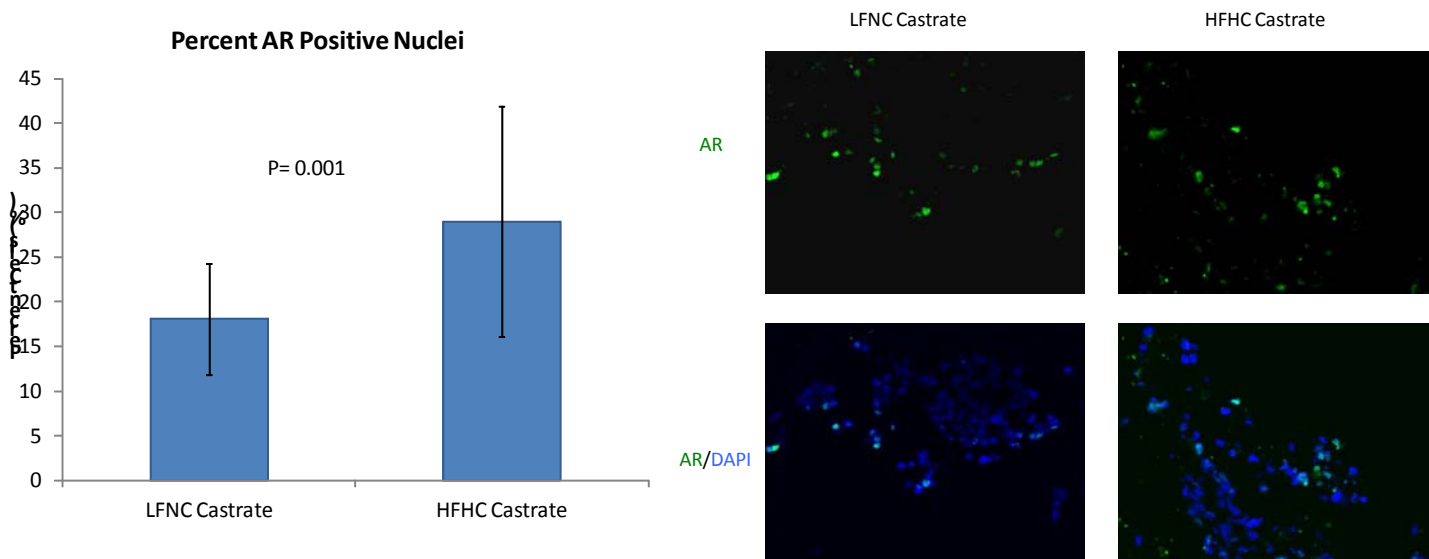


Figure 3. Analysis of nuclear AR expression in PCa tumors. LNCaP tumors were implanted in male mice fed either our LFNC or HFHC diets, and were subsequently castrated. At the end of 6 w post castration the mice were sacrificed and the tumors analyzed by immunohistochemistry for expression of the AR. Right panels are representative AR staining (green) with DAPI counterstaining (blue). Left panels are the quantification of this staining with the data plotted as % positive nuclear staining cells vs. diet group \pm SD. $n = (22)$ 20x images/group. The Student's t test was used to determine significance, $p < 0.05$ is considered significant.

Hyperinsulinemia is not responsible for diet effects on tumor growth. In our experimental design 6 w old mice have their baseline cholesterol levels normalized by feeding them a low fat, no cholesterol (LFNC) diet for 2 w. The animals are then randomized into our unique diet scheme. The basic design of our diet approach uses a defined low fat/no cholesterol [LFNC—equivalent of a normal chow diet which typically contains nominal (2 parts/million) cholesterol] and a high fat/high cholesterol diet (HFHC, w/o sodium cholate). The diets are

balanced in micronutrients on a per-calorie basis (see Table 2), permitting us to use the diets isocalorically. Different gram amounts of each diet are fed to the mice, fixing the amount of calories/mouse. Our HFHC diet does not make mice obese, in fact they weigh the same as the mice fed the LFNC diet (Fig 4), does not raise triglyceride (TG) levels in mice (not shown), but does raise cholesterol levels significantly⁽¹⁸⁻²²⁾ (Fig 5). The diets are used with and without ezetimibe, a cholesterol reducing drug yielding 4 base diet groups: 1) LFNC; 2) LFNC + ezetimibe (30 mg/kg/day); 3) HFHC; and 4) HFHC + ezetimibe (30 mg/kg/day). Critical comments concerning our analysis of the effect of diet on tumor growth suggested that hyperinsulinemia could be contributing to tumor growth and might represent a substantial confounder in our analysis. Although, we have previously measured insulin levels in our mice, and

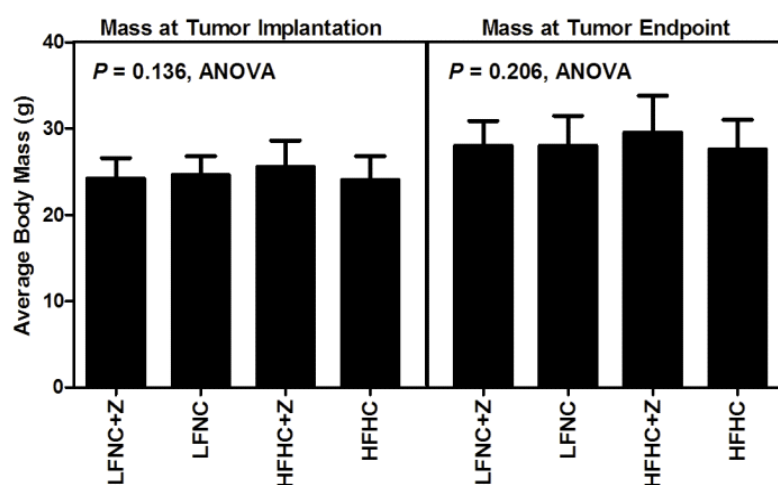


Figure 4. Body Mass. There was no effect of diet regimen on body weight. Average body mass is plotted as mass (g) \pm SEM vs. diet group. There was no difference in animal body mass at the time of tumor implantation, ($P = 0.135$, ANOVA), and no differences in body weight between groups were observed throughout the study as indicated by the average body weight at tumor endpoint pre-sacrifice ($P = 0.206$, ANOVA), $n = 27-30$ animals/group

found no significant difference in insulin levels in the different diet groups (Fig 6), we had not rigorously

determine whether insulin was contributing to tumor growth in a manner that could withstand rigorous review. To determine whether insulin was contributing to tumor growth we combined all the mice from all the diet groups and divided them into 2 groups based solely on serum insulin levels, < 1.0 ng/ml and ≥ 1.0 ng/ml (Fig 7). As demonstrated there is no relationship between serum insulin levels and tumor volume. In contrast when all the mice were similarly divided by cholesterol level (<124 vs. ≥ 124 mg/dL) significant differences in tumor volume were found between the two groups (Fig 8). In total, this analysis supports a specific effect of hypercholesterolemia in our model and little/no role for hyperinsulinemia.

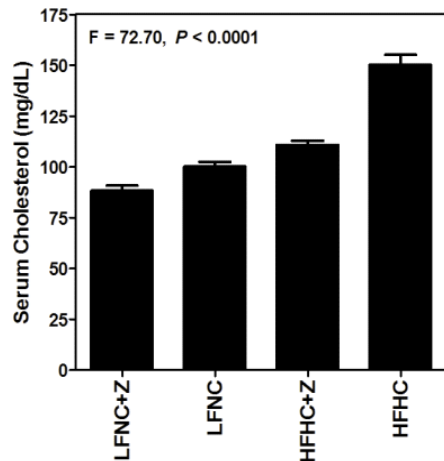


Figure 5. Serum Cholesterol Levels. Mice were fed either high fat, high cholesterol (HFHC) or a low fat, no cholesterol (LFNC) diet \pm ezetimibe (Z) and bled for cholesterol determination every 3-4 weeks throughout the course of the study. Serum cholesterol levels just prior to the first measurable tumors appeared are presented as serum cholesterol (mg/dL) vs. diet cohort \pm SEM. All groups were statistically different from one another ($F = 72.70$, $P < 0.0001$) and this difference was maintained throughout the study. An analysis of variance (ANOVA) was used to determine statistical significance. Values < 0.01 were considered significant, $n = 28$ /group.

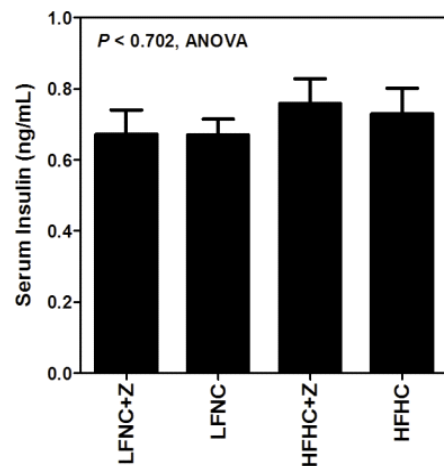


Figure 6. B. Serum Insulin Levels. Serum insulin levels are plotted as insulin levels (ng/ml) \pm SEM vs. diet group. There was no difference in serum insulin levels between diet and ezetimibe (Z) treatment groups at sacrifice ($P = 0.278$, ANOVA), $n = 15$ /group.

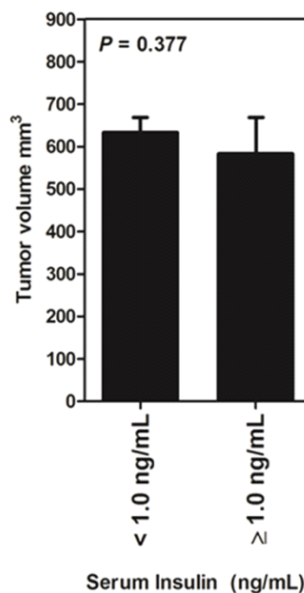


Figure 7. Serum insulin levels are not associated with tumor volume. Animals with the highest serum insulin levels, defined as serum insulin levels two standard deviations about the mean, blinded to treatment group, a value of ≥ 1.0 ng/mL, had a final average tumor volume that was the same tumor volume of animal with normal serum insulin levels of < 1.0 ng/mL ($P = 0.377$, t-test), $n = 8-49$ animals/group. A Pearson Correlation analysis blinded to treatment group showed that no association exists between serum insulin levels and tumor volume (Pearson $r = -0.140$, $P = 0.30$).

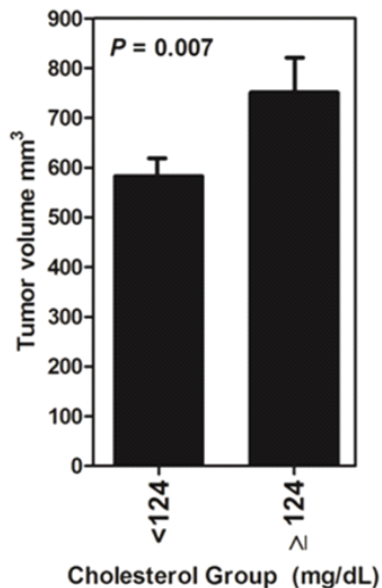


Figure 8. A. Tumor Growth is Correlated with Cholesterol Level. Average final tumor volume (mm³) ± SEM vs. serum cholesterol group is plotted. Hypercholesterolemic mice, blinded to drug/diet group, were defined as animals possessing serum cholesterol levels of 2 SD above the mean of mice on a normal diet (≥ 124 mg/dL cholesterol). These hypercholesterolemic mice demonstrated a significantly greater average terminal tumor volume than animals with cholesterol levels < 124 mg/dL cholesterol, (751 ± 372 mm³ vs. 583 ± 22 mm³, P = 0.007, t-test). The mean difference between the two subgroups was 168 mm³ and the 95% confidence interval is 48 to 298 mm³ higher among animals with cholesterol ≥ 124 mg/dL. n = 25 mice in ≥ 124 mg/dL cholesterol group & 81 mice in < 124 mg/dL cholesterol group. In an analysis blinded to treatment, the Pearson correlation between serum cholesterol and total tumor volume is positive (Pearson r = 0.23, P = 0.018) and clearly reveals an association that animals with higher serum cholesterol tend to have high tumor volumes.

Key Research Accomplishments:

- Demonstrated that hypercholesterolemia in castrated mice leads to decreased tumor levels of CYP17 substrates.
- Demonstrated that hypercholesterolemia appears to increase the tumor levels of androgens in castrated mice, similar to what we have shown in intact mice.
- Demonstrated that hypercholesterolemia increases the level of tumor nuclear androgen receptor in castrated mice.
- Further demonstrated the reproducibility of our diet model in raising serum cholesterol levels without effects on body mass, triglyceride levels, or insulin levels.
- Further demonstrated that hypercholesterolemia is associated with increased growth of tumors.
- Rewrote the SOW for the grant in order to allow for the collection of human prostate tissue from the Durham VA only.
- Produced an approvable IRB protocol to allow the collection of prostates from African American and Caucasian men requiring radical prostatectomies for prostate cancer.

Reportable Outcomes:

- 1) Created protocol to establish a prostate cancer tissue collection indexed with serum cholesterol values.
- 2) Began the analysis of steroid/androgen levels in prostate tumors implanted in xenograft mice, with preliminary evidence suggesting that while the intratumoral level of CYP17 substrates are lower in mice fed a HFHC diet, the intratumoral level of androgens is higher in these mice.
- 3) Began the analysis of nuclear androgen receptor (AR) levels in castrated mice fed a LFNC vs. HFHC diet, revealing significantly higher levels of nuclear AR in castrated mice fed the HFHC diet.
- 4) Conclusively demonstrated that hyperinsulinemia is not the cause of increased tumor growth in mice fed our HFHC diet.
- 5) Dr. Solomon sat of a Minority Men's Health Initiative Study Section due in part to the work supported by this award.

- 6) Dr. Solomon is the author and consultant on a study testing the effects of simvastatin neo adjuvant therapy on the outcome of definitive proton beam therapy treatment for PCa to be conducted at Hampton University.
- 7) Kristine Pelton was given a promotion based in part on her efforts supported by this award.

Conclusion:

We have begun the analysis of tumor xenografts from castrated mice fed defined diets and found that, as hypothesized, a high fat, high cholesterol ‘Western diet’ appears to contribute to intratumoral androgen synthesis based on: 1) the lower levels of CYP17 substrates in the tumors of castrated mice fed the HFHC vs. LFNC diet, 2) the higher levels of androgens in the tumors of castrated mice fed the HFHC vs. LFNC diet, and 3) the increased levels of nuclear localized AR in the tumors of castrated mice fed the HFHC vs. LFNC diet. We have also created a human subjects protocol that has been approved by the Durham VA and the DoD. We anticipate collecting human prostates for analysis in the next few weeks and begin to generate the data required to address the question of whether there are differences between Caucasian and African-American men in their levels of intratumoral androgen and whether high levels of serum cholesterol influence these levels.

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